

External Peer Review Ethylene Glycol Butyl Ether (EGBE)

FINAL REPORT

**Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency**

**Prepared by
ORISE IRIS Technical Assistance Team
Oak Ridge Institute for Science and Education
Oak Ridge Associated Universities**

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EXTERNAL PEER REVIEWERS

Henry C. Pitot, M.D., Ph. D., Chair
McArdle Laboratory for Cancer Research
University of Wisconsin

Xi Haung, Ph. D.
Nelson Institute of Environmental Medicine
New York University School of Medicine

Lisa Kamendulis, Ph. D.
Indiana University School of Medicine

Hazel B. Matthews, Ph.D.
Matthews Toxicology Consulting

Abraham Nyska, DVM
National Institutes of Health

Torka Poet, Ph.D.
Pacific Northwest Laboratories

Frank Welsch, Ph. D.
Orbitox

PEER REVIEW PROJECT

Jeff Gift, Ph.D., Project Manager
U. S. Environmental Protection Agency

Leslie Shapard, Peer Review Manager
Oak Ridge Institute for Science and Education

U. S. EPA
Ethylene Glycol Butyl Ether

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(EGBE)
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Introduction

This document is the report of the May 2004 external panel review of an Interim Final position paper titled "An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether (EGBE)" that was developed by EPA's National Center for Environmental Assessment (NCEA) in support of the Agency's Office of Air Quality Planning and Standards (OAQPS). This Interim Final version takes into account comments received from an internal peer review by EPA scientists and an external "letter" review by experts in areas relevant to the toxicology of this chemical (U.S.EPA, 2003).

External panel peer reviewers responded to the charge questions, listed below.

Charge Questions

1. EPA's 1999 IRIS assessment and Interim Final position paper place particular focus on hemangiosarcomas of the liver observed in male mice exposed to EGBE because this tumor type was increased over both concurrent and historical controls and because a relatively detailed mode of action involving EGBE has been proposed in the literature. In the position paper, EPA describes a mode of action for this tumor related to iron deposition following hemolysis. However, EPA stated that a definitive determination regarding the role of BAL could not be made and that "additional research (e.g., verification of existing PBPK modeling results and improved genotoxicity assays) would assist the Agency in making a more informed decision concerning the potential for BAL to contribute to the adverse effects seen in animals following EGBE exposure and use of the proposed nonlinear assessment approach." Considering the recent technical submissions (see CD) made in response to EPA's November, 2003 proposed rule:
 - a. Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced liver tumors?
 - b. Is the current information adequate to support the mode of action described in the position paper for the EGBE induced formation of hemangiosarcomas in male mice and the potential relevance of this finding to humans?
 - c. Does the available information support a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hemolytic effects in humans would prevent the formation of liver tumors in humans)?
2. NTP (2000) also identified forestomach tumors in female mice following EGBE exposure. In its position paper, EPA describes a mode of action for this tumor related to retention in the forestomach, metabolism to BAA, irritation and cell proliferation. However, EPA again stated that a definitive determination regarding the role of BAL could not be made and that "additional research (e.g., verification of existing PBPK modeling results and improved genotoxicity assays) would assist the Agency in making a more informed decision concerning the potential for BAL to contribute to the adverse effects seen in animals following EGBE exposure and use of the proposed nonlinear assessment approach." Considering the recent technical submissions (see above) made in response to EPA's November, 2003 proposed rule:
 - a. Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced forestomach tumors?
 - b. Is the current information adequate to support the mode of action described in the position

paper for the EGBE induced formation of forestomach tumors in female mice and the potential relevance of this finding to humans?

- c. Does the available information support a nonlinear cancer assessment approach for the female mouse forestomach tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hyperplastic effects in humans would prevent the formation of gastrointestinal tumors in humans)?
3. In addition to preparing written comments which address the issues above, feel free to provide any additional comments or recommendations you feel are important to this assessment. If your suggestions include references to published material, please provide a photocopy of the cited material. Feel free to make legible notations in the page margins and return those annotated pages with your written comments. If your comments are limited to particular sections of these documents or to particular issues, please indicate clearly the limitations of your review.

**Review Meeting on the EPA 2003 Interim Final Position Paper entitled “An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether”,
Held at the Environmental Protection Agency, Research Triangle Park, NC,
May 19, 2004**

Panel Members

Henry Pitot, University of Wisconsin, Madison, Chair
Xi Huang, New York University
Lisa Kamendulis, Indiana University
Hazel B. “Skip” Matthews, Matthews Consulting
Abraham Nyska, NIEHS
Torka Poet, Battelle Pacific Northwest National Laboratories
Frank Welsch, Orbitox

Others in Attendance

Jeff Gift, National Center for Environmental Assessment, RTP
Bertram Price, Price Associates, Inc.
Chon Shoaf, National Center for Environmental Assessment, RTP
Tipton Tyler, Health Studies Management and Consulting

Introduction

Henry Pitot, Chair of the Review Panel called the meeting to order at 9:07 AM. Leslie Shapard of the Oak Ridge Institute for Science and Education (ORISE) made announcements about safety procedures and conflict-of-interest certification. ORISE is responsible for collecting information from potential members of the review panel and resolving identified personal conflict-of-interest and bias issues. The seven members of the panel completed conflict-of-interest disclosure forms which were examined by ORISE. ORISE has certified that those seven reviewers, to the best of it's knowledge and belief, there are no identified relevant facts or circumstances that could give rise to a conflict-of-interest beyond those that follow. ORISE has determined that there were two responses to the questions that they believe should be disclosed: Kamendulis has been involved with research on 2-butoxyethanol sponsored in part by the glycol ether panel of the American Chemistry Council. She was co-investigator; Jim Klaunig was PI. She has co-authored several publications on the research.

In addition, Poet conducted research sponsored by the American Chemistry Council on EGB kinetics in mice. Her work in this area has been published. She has on-going work with related chemicals, also sponsored by ACC. The reviewers were asked if they have anything else to disclose related to conflict of interest or bias. All panel members responded with a negative.

Shapard said that while consensus is not required on the issues, the Panel Chair would seek the collective opinion of the panel wherever possible. The EPA Project Manager, Jeff Gift was present for the purpose of providing clarification and answering any questions. The panel members would have an opportunity to revise their responses after the meeting and to have them back by May 28th.

Dr. Pitot thanked everyone for attending. He suggested not going around the table and having the panel introduce themselves to each other and the audience as everybody introduced themselves before the meeting.

Pitot then presented and summarized slides related to the review including topics which the information the EPA felt they needed in relation to the liver and forestomach neoplasms in male and female mice, respectively. Pitot made the following presentation:

Slide 1:

Information Needed by EPA in Relation to Significant Increases in Liver Neoplasms and Forestomach Papillomas in Male and Female Mice Respectively

- Verification of existing PBPK modeling results.
- Potential relationship of the "genotoxic" effects of BAL *in vitro* on the adverse effects seen in animals following EGBE chronic exposure.

Slide 2:

Specific Questions to be considered

- Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced liver and forestomach tumors in male and female mice respectively?
- Is the current information adequate to support the mode of action described in the position paper for the EGBE induced formation of hepatic hemangiosarcomas and forestomach tumors in male and female mice respectively and the potential relevance of this finding to humans?
- Does the available information support a nonlinear cancer assessment approach for the development of male mouse liver tumors and for the development of forestomach tumors in the female mouse observed following EGBE exposure?

Slide 3:

BAL Genotoxicity Studies

Direct Clastogenesis *in Vitro* (published studies)

Sister Chromatid Exchange *in Vitro* (unpublished studies)

Pitot said that the Comet assay results would fall under the direct genotoxicity method.

Indirect 8-Hydroxydeoxyguanosine Formation in Endothelial Cells

Slide 4:

Proposed Pathogenesis of Hepatic Hemangiosarcomas and Forestomach Tumors in Male and Female Mice Respectively

Pitot said that the panel would consider liver neoplasms in the morning and try to come to a consensus by the end of the morning. In the afternoon, the panel will consider forestomach neoplasms and try to come to a consensus by that time. He hoped that there will be enough time to have public comments, before the panel tries to summarize.

- Role of Hemolysis and Iron Deposition in Liver – Mechanisms
- Role of Gastric Retention of EGBE, Inflammation and Cell Proliferation/Hyperplasia in Forestomach Epithelium – Possible Role of Tumor Promotion
- Discussion of report by Deisinger and Boatman

Slide 5:

Nonlinear Cancer Assessment Applicable to Development of Liver Tumors and Forestomach Tumors in Male and Female Mice Observed Following EGBE Exposure Respectively?

YES

Indirect Effect of Iron Deposition in Liver

Gastric Retention of EGBE Doses

Cell Proliferation/Hyperplasia Induced in Epithelium of Mouse Forestomach

NO

Possible Genotoxic Effects of BAL Metabolite as Demonstrated by Clastogenesis *in Vitro*

Pitot asked the panel members to go around the room and give a summary of how they see the three questions related to the liver neoplasms.

Huang said the position paper is very well written and has lots of information that is interesting. He said that the first point is that there is enough information to support an informed decision concerning the significance. It is difficult to consider the DNA-protein crosslinks. Formaldehydes are very good agents in inducing DNA-protein crosslinks and there is enough literature to support that. DNA protein crosslinks could impair DNA repair activity. That is the point that should be addressed. In another point regarding susceptible populations, he had another concern about Asian people. Among Japanese, Chinese, and Koreans they are about 50% aldehyde dehydrogenase deficient. By that he meant that those people who inhale EGBE, are they going to accumulate BAL? He didn't like the fact that this has not been determined. Once you have ALDH deficiency, you may have an accumulation of BAL and this population may be more susceptible compared to other populations.

Huang agreed with the second question. He thinks there is a fair amount of evidence that BAL can cause hemolysis and because the BAL-induced hemolysis is an indirect mechanism, the mode-of-action has no relevance here. Again, since he has been working with iron he has found that acidic pH normally can cause iron release from transferrin. Since BAA is acidic, high concentrations may cause iron release from transferrin. Transferrin in the serum is about 10-20 umol. So if you have an acidic pH from BAA, there may be iron release, and free iron can cause excessive damage. This damage is not only limited to hemolysis. He agreed with question 'c'.

Kamendulis started with a couple of points on question 1a. She thought that in light of the new data that was presented that the pharmacokinetic study and the quantitative measurements indicate how much BAL should be produced and the levels are quite low. In contrast with the studies that were done with the genotoxicity of the BAL metabolite, it took excessively high doses of the BAL metabolite to induce any changes. With these two bits of information together it seems unlikely that the BAL metabolite can contribute to inducing the genotoxicity effect. She thought that the BAL metabolite would have a negligible effect.

As far as question B is concerned she addressed some other components that might be included in the proposed MOA. They do not negate what she would consider the two steps, that being the induction of hemolysis as an important component but that the induction may not solely be an iron issue. A key component may be the action of the Kupffer cells, which can release reactive oxygen species and cytokines that may impact growth. Growth of course, is required for the induction of the hemangiosarcomas. She thought that one of the hinge points was lack of any findings of iron within the endothelial cells. This alternate hypothesis with alternate steps involving the Kupffer cells may negate the necessity to find iron in the target cells. The activation of Kupffer cells could be involved in the critical step. The activation may occur by damaging the blood cells resulting in the release of ROS. While iron is a component, it may not be the only component in the pathway. BAL may trigger the initial hemolytic event. She agreed that there is enough information to support the proposed mode-of-action. She offered alternate steps that may be involved in the mechanism of action but not necessarily alter the proposed mode-of-action.

As for question "C" she had an issue with the language of how the question was posed. Is it reasonable to expect that prevention of hemolytic effects in humans would prevent the liver tumors in humans? She indicated that prevention is more of an active process. If the question is: are the effects not observed in humans, i.e., the induction of the hemolysis isn't achieved by EGBE then will we not see the proposed MOA? The answer is yes. She objected to the language as to how the question was written. If hemolysis was prevented in a mouse, then it could be assumed that the lesions would not appear as well. She was looking for a clarification on what the intent of that question was. She also asked for clarification on what types of tumors were relevant for this discussion. The document states in a number of places that the focus is carcinomas. She thought the questions were drafted with one tumor in mind. She thought that question 1a and b refer to liver neoplasia in general which would encompass both tumor sites whereas 1b is specific to hemangiosarcomas. So to be complete with her answer, she wanted to make sure that the question was drafted accurately.

There was some discussion as to what tumor types should be considered. Pitot said that regulatory agencies lump adenomas and carcinomas together, but here the panel was focusing on hemangiosarcomas. Kamendulis suggested the language be changed to define the tumor types that were under discussion.

There was a brief discussion about the "prevention" issue and whether the wording in the document should be changed.

Matthews made comments on the document. For the first question, in his opinion there is enough information in the summary document, as we can expect to have on any particular chemical to determine risk. He said that we certainly know that the parent compound is metabolized and the entire metabolism goes through the aldehyde. However the metabolism of the aldehyde to the acid is more rapid than the formation of the aldehyde. Therefore the concentration of the aldehyde remains quite low. In reading his own comments in advance it seemed that experiments could be performed using human hepatocytes from aldehyde dehydrogenase-deficient populations to determine if there was an accumulation of BAL. So if additional work was done, the panel might want to discuss those experiments. He indicated that BAL might be a mutagen at very high concentrations but certainly those concentrations are not important in a living organism. The comet assay indicates that BAL is not reacting with DNA.

For the B question, again he thought the answer was "yes". We know that the acid is the hemolytic agent and we know that the accumulation of heme accounts for the adverse effects in the liver that probably lead to carcinogenesis. He indicated that Kamendulis discussed that aspect very well.

In question C, does the available information support nonlinear cancer assessment? And he answered yes. The hemangiosarcomas were observed at the highest dose and only in male mice. There were a number of explanations that might account for that the tumors the least of which was the fact that the mice were dosed with higher doses than rats and mice inhale more air per gram body weight than rats. The male mouse liver is also susceptible to liver tumor induction.

Huang brought up the existence of the ALDH-null mice.

Pitot agreed with the previous speakers that BAL is negative in standard mutagenesis assays. He indicated that the original references in the review by Elliot and Ashby couldn't be reviewed by him in time for this meeting. High levels of BAA which are in the millimolar or 1/10 millimolar concentration ranges are clastogenic. The B79 cells are already chromosomally abnormal and chemical treatment may easily change the karyotype of the cells. This does not occur in the primary lymphocytes. Those experiments are the most significant. For all the experiments (except for the 600 mg given as very short dose), the levels of BAL were still well below the lowest level that showed any effect. The evidence indicates that BAL is not mutagenic from the multistage viewpoint and that it is not an initiator. It does not by itself have any promoting activity. He said that you cannot dismiss the possibility that BAL should be considered a progressor agent. In fact it does have some clastogenic effect. He said that you don't really know the dose relationships here. The differences are nearly an order of magnitude and in some cases two from the effects that are seen in the lymphocytes compared to the levels of BAL that are produced in the liver. BAL is not a significant factor.

On the second question, he thought that an indirect mechanism is related to the hemangiosarcomas and not to the liver tumors themselves. Human heterozygote carriers of the ALDH gene may be a little more susceptible to exposure but genetic background is probably not a factor.

On the third question, even if all the arguments are made that the compound is slightly clastogenic, you are still going to get a nonlinear effect. All data argues that the mechanism is an indirect effect.

Nyska discussed the correlation between hematochromatosis in the liver and the frequency of hemangiosarcomas from a number of NTP studies and noted that there is a highly significant correlation. This relationship is observed only in male mice and not in female mice. He said that female mice may be more protected from oxidative stress because of the higher concentrations of anti-oxidants in liver. This is

a mouse phenomenon. He was very interested in the acute events that occur in this example. Studies have been done that show that human erythrocytes are less sensitive to damage.

He also agreed with the mode of action and the nonlinear effects of this compound.

Poet made the point that although the aldehyde is genotoxic in vitro, the levels used to achieve genotoxicity in vitro are never achievable in vivo. As far as the effects in Asians with decreased ALDH activity, liver blood flow will far outweigh any decreases in the activity of ALDH because ALDH is a low affinity enzyme with a moderate capacity. If you have that capacity, you will have the same affinity. She indicated that the knockout mouse studies will not be very informative. BAL might be genotoxic but the level will never be that toxic because of the metabolism effects. If you agree with the hemolysis, then the mode of action beyond that will not be that important an issue. She agreed with a nonlinear mode of action.

Welsch agreed that the background data of the document was correct. He agreed with question A. The comet assay showed no DNA interactions at subcytotoxic concentrations. He likened this situation to 2-methoxy acetaldehyde (2-MALD) and 2-MAA that his lab worked on for some years. Interest in this compound faded away because of reformulation of glycol ether products that contained those glycol ether congeners with high reproductive and developmental toxicity hazard potential based on animal studies. Among them was ethylene glycol monomethyl ether (EGME) which upon metabolism gives rise to the intermediate 2-MALD and then to 2-MAA. The kinetic studies with EGME showed that one could never achieve a high enough BAL concentration that has any relevance to the issue at hand. There is enough information to make judgment on point a. He agreed with questions B and C.

Pitot opened up a general discussion of the points discussed. Regarding the ALDH knockout, he raised the point that to mimic the Asian response the experiment should be done with the heterozygotes. However, this tells us nothing about the affinity of the enzyme for the compound. Usually you have too much compound around. On the other hand, many Asians are sensitive to ethanol. If you are feeding astronomical levels of material, you may see some effects. This pertains to the question of variants in the population in regard to ALDH.

Huang made the point that native Indians are also deficient in ALDH activity.

Kamendulis asked whether you can determine the effect of a deficient enzyme on the BAL produced from EGME exposure in the ALDH knockout mouse. Is it possible to reach the levels of BAL necessary to achieve the genotoxic effect?

Poet noted that this would be possible but that nobody has done it is because there is an excess of enzyme. The blood flow is the limiting factor even by cutting the enzyme levels in half.

Matthews asked if it is possible to characterize human hepatocyte ALDH levels and using these cells, address the same question of BAL involvement as proposed for the knockout mice?

Pitot made the point that human hepatocytes are quite different than the intact liver. This is the same for liver slices. The knockout mice would be the best model.

Huang noted that the experiment would take a long time.

Pitot and others discussed that the PB-PK model could be used very fast to determine the role of deficient ALDH on BAL levels. Poet mentioned that she might be able to do it before the final write-up.

Huang noted that he has the hemochromatosis knockout mice.

Poet noted that if you do not see much difference in the metabolism of ethanol in the ALDH knockout mice you are not going to see any difference for EGME.

Pitot noted that the hemochromatosis knockout mouse model might not be appropriate as the concentrations of material that would produce hemolysis would never be high enough. However this may be the only model to use given the segment of the population that we discussed.

Shapard noted that the notes of the meeting would be made available to the panel members to verify that the report is reflective of the discussion that occurred.

Pitot called a break at 10:05 AM.

Pitot reconvened the meeting at 10:32 AM and asked the panel members if any questions had come up during the break. As no questions came up, he said that they can now start to consider the charge to the reviewers and come to a consensus for questions A, B and C for the hemangiosarcomas in the liver. He noted that it is important to specify the tumor type as the hepatocellular adenomas and carcinomas are not relevant as shown by the NTP studies. He suggested that in the first part they substitute liver tumors with hemangiosarcomas. Pitot outlined the procedures for coming to a consensus.

Matthews moved that enough information is now available to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE-induced hemangiosarcomas.

Kamendulis seconded.

Discussion of the motion:

There was a brief discussion as to how to include experiments that might add to the understanding of the MOA of the compound. No panel members wanted to make an addendum to the motion. Pitot suggested that after questions A-C are voted on, that by consensus they could agree to add suggestions for additional experiments.

A vote was taken on A. All votes in favor.

Pitot suggested that EPA might want to consider additional experiments to address the potential sensitive subpopulations to BAL as pertaining to the ALDH knockout mice and the use of PB-PK modeling to be performed by Poet in an effort to examine aldehyde dehydrogenase deficient populations including Asians and Indians.

Kamendulis made the motion that adequate information supports the mode of action described in the position paper for the EGBE-induced formation of hemangiosarcomas in male mice and the potential relevance of this finding to humans.

Poet seconded.

Pitot asked for comments from the audience.

There was a discussion as to whether the carcinomas in the liver should be considered. Pitot suggested that an addendum be considered that the liver carcinomas are not significant. Nyska indicated that there is very little data to support an increase in the carcinomas in the mouse from the NTP studies. He said that the NTP report indicated some evidence for an increase in the carcinomas in the mouse after exposure. He agreed to look into the data while the rest of the panel continued the discussion.

A vote was taken on motion B. All voted in favor.

Shoaf asked about the sensitive populations and whether it was inconsistent with the motion that was passed, i.e., there is enough information to vote on A but more experiments would be required to define the sensitive subpopulations.

Pitot indicated that it is up to the EPA as to what they do with the proposed experiment put forward by the panel and this information will be put in the summary. Others indicated that there is some confusion that by saying yes to A and then to say there are additional experiments to do will add confusion to what the panel is saying. There was general agreement that the proposed experiments should be put at the end of document as “informational points” or “informational material”.

Nyska read from the NTP report as to the hepatocellular carcinomas in the mice after exposure to ETBE. He didn’t think that the incidence was significant because the incidence was the same in the controls and treated animals.

Kamendulis indicated that there is no reason to believe that the mode of action is not relevant to the carcinomas.

Pitot indicated that in question C, the question was about liver tumors and not specifically about hemangiosarcomas and thus should be changed to reflect the discussion.

There was additional discussion about the word “prevent” in question C as to whether the word was appropriate or not and whether EPA would allow the question to be changed.

Kamendulis asked Gift as to whether the wording about the tumors in A and B should be the same. She asked what was the intent of the EPA for the panel to consider one tumor type or all tumor types? There was discussion to leave it as is and to consider both types of tumors.

Pitot brought up the issue of the word “prevention”.

Matthews suggested putting in “lack of” in place of “prevention”. There was agreement from the rest of the panel.

Matthews proposed the motion that the available information supports a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure, and i.e., it is reasonable to expect that a lack of hemolytic effects in humans would prevent the formation of liver tumors in humans.

Kamendulis seconded.

Discussion of the motion:

Poet made the point that by inhalation exposure the dose could never be high enough to achieve the concentration of BAL to cause effects in the liver.

There was a discussion as to whether “i.e.,” should be removed “and “therefore it is” added. There was a motion to make the above changes and a second.

The modified motion after the discussion now read: the available information supports a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure, and therefore it is reasonable to expect that a lack of hemolytic effects in humans would prevent the formation of liver tumors in humans.

A vote was taken on question C. All voted in favor.

Pitot suggested that the panel discuss the additional experiments to be added as informational points at the end of the day after voting on the additional questions in the afternoon.

Shoaf asked for clarification on question A.

There was limited discussion on hemolytic effects, the definition of mode of action vs. mechanism of action, release of iron from transferrin by BAA, and the use of castrated male mice to determine the role of

testosterone and estrogens in the male mouse liver tumor response. No further recommendations were made to incorporate these discussions into the information at the end of the report.

Pitot adjourned the panel at 11:30 for lunch.

Pitot reconvened the panel at 12:33 PM and directed the members to paraphrase their comments made to the questions in the charge pertaining to the female mouse forestomach tumors.

Welsch began the comments by saying the additional work that was performed was responsive to the issues raised in the 2003 EPA document. He agreed with question A based on the comet assays and the pharmacokinetic data performed at Eastman Kodak and the PB-PK work at Pacific Northwest Laboratories. He said that multiple routes of administration of EGBE following its fate in animals did provide new information for him to allow a decision concerning stomach tumors. It was important to consider the fact that the forestomach has no functional or structural counterpart in humans. The available data supports the position paper.

For question B, he said that the current information is adequate.

For question C, he said that the question was worded wrong: the issue was not female mouse liver tumors but female forestomach tumors. The scientific evidence indicates that the irritation leads to the hyperplastic response. The fact there are differences in anatomy between mice and man and resident time of foodstuff makes it highly unlikely that the tumors could occur in man.

Poet agreed with questions A-C, that there is enough information to support the mode of action submitted in the paper.

Nyska gave a pathologists perspective to the mechanism of action of EGBE for forestomach tumors. Inflammation was only seen in females and not in males. The hyperplasia was higher in the females. Ulceration was more intense and had higher incidence in the females than males so there was a correlation between the damage and the development of the tumors.

Pitot said the first question pertains to the forestomach and the liver tumors and the panel is going to have to come back to this point. His response to question A is that enough information exists. He indicated that the panel responses have been yes but that they should carry the response farther by saying that the answer is "yes" and it does not factor in. He indicated that the wording of question A is not very solid but that the panel can return to this. For the second question, he agreed that the panel is really dealing with papillomas in the forestomach. The extent of cell proliferation and hyperplasia can explain the female tumor response, because the cell proliferation and hyperplasia does not occur in the males. This doesn't tell what this toxicity is, but allows one to make a conclusion with respect to the effect of cell proliferation and hyperplasia on tumor induction. The data indicates that EGBE metabolite is acting as a promoting agent through this indirect mechanism, causing cell proliferation and hyperplasia and causing growth of spontaneously initiated cells. One thing that wasn't done was a stop experiment to see if the papillomas regress. He found an article on methylacrylate, a more potent compound than BAL and the forestomach tumors regressed after stopping exposure.

For the third question, he argued that the answer is yes given the fact that promoting agents clearly act by a nonlinear mechanism. In addition in humans there is no structure that is equivalent to the mouse forestomach. The problem in humans is the increase in the incidence in Barrett's tumors in the esophagus that one finds but it is an entirely different mechanism. The mechanism is based on regurgitation of acidity in the stomach which causes chronic inflammation and proliferation in the gastroesophageal junction and leads to the same sort of response. If the regurgitation can be corrected, the Barrett's disappears. He said that the mechanism is a little like the methylacrylate mechanism. If you have too much damage, you go to a point where the features of the disease will not disappear because the cells have transited from the point of promotion to progression. The risk for developing Barrett's has very little to do with anything that is

coming in from the environment except for the fact that some of the patients eat too much. For those reasons, he argued that the data supports a nonlinear approach to this lesion.

Matthews said that he responded “no” to question 2A because of the wording of the question. He didn’t believe that we can differentiate the possible irritation of the forestomach by EGBE, BAL or the acid to which it is metabolized. It doesn’t mean that he doesn’t think that the administration EGBE did not induce the forestomach tumors.

In question 2B he thinks that the current information is good but could be improved with additional experiments. There is a need to more clearly separate the forestomach and the gastric stomach than was described in the material provided. The material indicated that there was not a clean separation between the two. However, he said that nothing will answer quite all the questions.

In question 2C he answered yes. He said that this situation is not unique to EGBE as other chemicals that cause forestomach tumors frequently do not cause tumors at distance sites but are associated with gavage administration. These compounds at lower doses do not induce forestomach irritation and associated tumors.

Kamendulis addressed question 2A by saying the kinetic information indicates that the aldehyde metabolite of EGBE will not be produced in sufficient quantities to play a role in the forestomach tumors in the female mice.

For 2B, she agreed. She said that as far as relevance to humans, because of PD and PK factors between species, the mechanism is not likely to occur in humans. There is sufficient information for the proposed mode of action for the mouse.

For question 2C, she answered yes. She objected to the use of the word “prevention” of the hyperplastic effects in humans for two reasons in this case: prevention being an active process and because humans do not have forestomachs which is addressed in the question. She wanted to talk about rewording of the question.

Pitot talked about the definition of the forestomach “equivalent” in the human. The gastroesophageal junction may be a more appropriate definition in this case, to be discussed later.

Huang discussed question 2B first. Humans have no comparable organs compared to the mouse forestomach. Because the mode of action in rodents is due to the disposition and retention of EGBE and BAA, the risk for humans is very minimal. His answer for question 2B was yes.

For question 2A, he was concerned about the ALDH deficient population. Do they have a higher accumulation of BAL in the GI system? His answer for 2A was no, there is not enough information to answer this question.

For question 2C, his answer was yes. When he read the position paper, he didn’t see which metabolite of EGBE caused the hyperplasia in the forestomach.

Poet said that the metabolite is probably BAA but did not see a definitive statement about that in the document. She suggested changing the 2C to a single sentence “and therefore” and adding “gastroesophageal junction”.

Gift pointed out that the conversion of EGBE to BAA is part of the mode of action discussed in the Agency’s interim final position paper, but didn’t believe the paper identified BAA as the sole cause of hyperplasia.

Kamendulis asked whether the panel was trying to pinpoint the cell proliferation to a specific chemical or we are getting cell proliferation subsequent to irritation and an inflammatory response.

Nyska said that irritation and inflammation are necessary for cell proliferation. There is only one compound, from his own experience (2,4 Hexadienal), that induces cell proliferation without irritation in the forestomach. The majority of forestomach carcinogens induce regenerative hyperplasia after irritation. He said that morphologically sometimes, the structure of severe regenerative hyperplasia and the papilloma are very similar.

Pitot said that if you eliminate the hyperplasia with cortisone in mouse epidermal carcinogenesis, a steroid, you would eliminate the papillomas. In that case, you are dealing with a secondary effect.

Pitot opened up the floor to a general discussion of the three points. He began the discussion by bringing up the wording of 2A and 2B to say that BAL is not a factor in the mechanism.

Matthews said that the compound is at extremely low concentrations, and is not reactive with DNA. However, he said that it would be difficult to say that it is not a factor. He thought that the administration of EGBE increases the forestomach tumors. He said that if the panel modifies 2A, we have to strike out the BAL metabolite altogether. We cannot conclusively say that BAL does not have any effects.

Poet brought up the point that there is very little of BAL to be causing effects. Even with polymorphisms in the aldehyde dehydrogenase, the Vmax is so high compared to alcohol dehydrogenase that the time of appearance will be so small and insignificant.

Poet said that the concentrations of BAL could never be achieved to induce the effects of BAL on genotoxicity that are seen in vitro.

Pitot said that you have the same problem in question 1A that the concentration of BAL to get the cancer is negligible.

Matthews said we couldn't conclusively say that BAL does not react with forestomach mucosa and cause the irritation.

Pitot said that the BAL metabolite is not a critical factor in the cell proliferation nor in the initial irritation. With respect to 1A, he felt there was enough information to support an informed decision. He asked whether the panel should add BAL is not a factor in carcinogenesis.

Matthews suggested that they leave 1A as is and modify 2A. There was a general discussion as to whether there should be any further modifications to 1A.

Pitot suggested that the panel should leave 1A alone. He said that the way the question is worded we really don't know if BAL is important in production of inflammation. Although the answer to question 2 is yes, the mechanism is indirect.

Matthews asked whether the BAL metabolite is important in this case because they administered EGBE and they got stomach tumors. Is it out of line to modify the question to strike BAL metabolite and address the question of EGBE?

Pitot said that it is, because of the importance of BAL as it was raised in the very first paragraph with respect to its genotoxicity. He argued that there was some minimal genotoxic effect of exposure, stimulating the cells to grow. With this we are getting into the toxicology of EGBE in the forestomach.

Gift wanted clarification from the panel as to whether BAL is significantly involved in the interaction with DNA and thus a linear model should be incorporated in the risk assessment.

Pitot said that there was no evidence for induced bacterial mutagenicity by BAL.

Matthews pointed out that many chemicals that are not mutagens cause forestomach tumors. He said that we cannot conclusively state that BAL is not a promoting agent as well.

Huang pointed out that there is evidence to show that BAL is not active genetically, mainly as a mutagen but that this is not necessary to cause genotoxicity. BAL may cause DNA-protein crosslinks.

There was a discussion as to whether BAL can cause DNA-protein crosslinks. There was agreement that at high doses it is possible but only at concentrations that will likely not be achieved.

Matthews pointed out that we cannot conclusively prove that there is no genotoxicity. He pointed out that in the liver the question we are talking about is mutagenesis and in the forestomach tumors we are talking about promotion. He asked whether we should focus on the genotoxicity of BAL or on the carcinogenicity of EGBE?

Pitot asked whether if we answered yes to the question that this would not rule out the fact that BAL plays a role in promoting the papillomas.

Matthews pointed out that any of the three compounds could be the promoting agent based on killing cells or stimulating inflammation.

Pitot said that we cannot change the wording of the question in this case, because BAL has to be in the question.

Gift said that you can change the wording to clarify the role of BAL in the carcinogenic process, i.e., its interaction with DNA.

Pitot said that most clastogens do not directly react with DNA, i.e., covalently.

Gift said that the intent is to determine whether there is a reason to use a linear response model. Alternatively the panel can take BAL out of the question.

Pitot said that the document focuses specifically on BAL and the panel should leave BAL in the question because that is what EPA is interested in. BAL is the only compound that has any genotoxic potential.

Pitot asked whether to change “metabolite” with BAL “genotoxicity”. The same thing that pertains to B and C, also does for A.

For question 2A, **Matthews** made a motion that enough information now exists to support an informed decision concerning the significance of BAL genotoxicity to the formation of EGBE-induced forestomach tumors.

Kamendulis seconded.

There was no discussion.

The panel voted on question 2A. The vote was unanimous.

For question 2B, **Kamendulis** made a motion that the current information is adequate to support the mode of action described in the position paper for the EGBE-induced formation of forestomach tumors in female mice and the potential relevance of this finding to humans.

Matthews seconded.

There was no discussion.

The panel voted on question 2B. The vote was unanimous.

There was a discussion as to how question 2C should be worded.

For question 2C, **Welsch** made the motion that the available information supports a nonlinear cancer assessment approach for the female mouse forestomach tumors observed following EGBE exposure, and therefore it is reasonable to expect that a lack of hyperplastic effects in the region of the gastroesophageal junction in humans would preclude the formation of gastroesophageal tumors in humans.

Poet seconded the motion.

There was a discussion as to the wording of the motion in terms of the gastroesophageal region and what type of tumors should be considered. Pitot pointed out that the incidence in humans of adenocarcinomas in the esophagus has dramatically increased during the last 3 decades but the incidence of squamous cell carcinoma has decreased. Pitot suggested leaving the question as is with the word 'tumor'.

There was agreement that the motion should be left as is.

The panel voted on question 2C. The vote was unanimous.

Pitot asked for additional comments/recommendations that would go into the report. There was agreement to leave it as comments and leave out recommendations. The first issue was to perform PB-PK modeling in the heterozygous ALDH knockout mice. The reason to do that was to use as a surrogate for Asian and Indian populations that have lower ALDH levels.

There was agreement not to propose to do any work with human hepatocytes.

Matthews made the comment about the housing of the mice in the EGBE studies in the NTP studies. Because the females were group housed, grooming of cage mates could contribute to the female-specific response in the forestomach.

Poet pointed out that the dose is probably too low for any effect in this case.

Shoaf pointed out that some of the experiments proposed could fall under the air toxics grant program.

There was a discussion as to the determination of the contribution of BAL in the ALDH knockout mouse experiment and the need to have a good inhalation facility. There was discussion that the analytical procedures to determine the levels of BAL are difficult because of the inherent levels of enzymes that can convert BAL to metabolites. The PB-PK models may be a more straightforward, easier way to determine whether the BAL metabolite could reach levels where effects could be observed. Poet indicated that she could do further work on determining the effect of lower ALDH enzyme levels on the levels of BAL using PB-PK modeling.

Tyler asked for clarification on the hemochromatosis mechanism.

Pitot said that hemochromatosis is due to increased transport of iron into cells. He said that the gene involved is not only in the intestinal tract but other cells including epithelial cells. If you have iron available from hemolysis, the iron is going to get into the cells. If you have the wild-type gene you can control how much iron is going into the cells. If you are not gating it with the gene, then you could have a minimal degree of hemolysis but get more iron into the cells.

Huang made some comments on hemochromatosis. He said that there is a point mutation in the HFE gene that causes the hemochromatosis. Cells transport iron through the transferrin receptor and HFE protein is bound to transferrin receptor. The transported iron goes into the endosomes of the cells. For the homozygotes, the percentage in the US among Caucasians is 0.5-0.8%; for heterozygotes it is 10-12%. There is one epidemiology study that shows an association between mutation in the gene and breast cancer and there is some information on mutations and liver dysfunction.

Tyler pointed out that they have discussed the significance of hemochromatosis within his group especially with a hematologist from Yale University. He has indicated that he has not found any literature concerning increases in hemangiosarcomas. He pointed out that Dr. Klaunig has done some work here. He asked whether there is something should we should be doing here with regard to EGBE?

Pitot indicated that the low level of hemolysis in vitro in human cells after exposure would not lead to the events discussed. In the HFE knockout mouse you would have to go to very low doses because if you go to high doses you will get hemolysis in both strains.

Huang said that if you knockout the HFE gene at 6 weeks old, transferrin is 100% recycled.

Tyler pointed out the fact that they have looked at other types of susceptible populations including sickle cell and some other types of red-type deficiencies and they are as resistant as normal blood cells in humans. They did not look at malaria patients.

Kamendulis said that the BAA is the active compound in the hemolytic effects and all data indicates that the millimolar levels required would not be achievable.

Poet indicated that there is no model to incorporate genetic changes into the traditional PB-PK model. You have to have quantifiable numbers to put in the model to have the computer model the effect. She was not certain how long it would take to build the model.

Kamendulis made the comment that the difference between sickle cell and hemochromatosis in which there is a fragility of the red blood cells with hemochromatosis but she didn't know if there was any difference in fragility. She said that there are two separate mechanisms: that one involves iron and one involves hemolysis.

Welsch posed the question that if the panel suggests additional experiments, will that hold up the EPA process or will the experiments be considered as separate issues for future consideration but will not hold up the process?

Price indicated that the panel could address the issue as additional experiments but make sure that the answers to the 6 questions is not contingent on these experiments being performed.

Pitot made the comment that perhaps additional comments should have a parenthesis that they are for the potential use by the EPA but not related to the questions raised.

Price suggested that the experiments proposed should be entirely separate from the answers to the 6 questions because the regulators will be looking at the document. If the group wants to write a proposal to the EPA to do these studies, they should do that but it should be separate.

Pitot suggested that the proposed experiments be left in the descriptions from the individual reviewers.

Price said that if the comments on sensitive subpopulations were to be left in the report then the regulators would look at that and try to incorporate that information into a risk assessment.

Poet asked if there was any quantifiable data as far as modeling goes of compounds in susceptible individuals that she could use as examples. Poet will put into her part a parallelogram approach to propose ethanol PB-PK modeling in sensitive and nonsensitive population comparisons.

Pitot proposed to leave it up to the committee to decide how to deal with these additional comments, possibly as caveats and asked how to deal with the issue of these additional comments and the implications the comments might raise.

Kamendulis proposed a motion to omit from the general comments, proposed experiments from the general report but include the proposed experiments in the individual reviewers comments.

Welsch seconded.

The vote was unanimous.

Poet said that if she could find actual metabolism data she could use the parallelogram approach to model the changes in metabolism of BAA and BAL in the populations. If she cannot find the data, she will change the Vmax of ALDH by 50%. She indicated a number of compounds that may be appropriate for the modeling studies.

Gift brought up the issue of exposure through grooming. How much additional accumulation do you get on their fur over a chronic study?

Poet indicated that the compound is fairly volatile, and there would be no appreciable exposure from the fur.

Gift mentioned radioisotope studies in which there was no difference in the accumulation on the fur from inhalation and nose-only studies. There was also accumulation of radioactivity in the salivary glands.

Poet noted the nose-only studies were positive pressure, and there could be some accumulation of the compound on the fur. She also added that she has some unpublished data that shows that more food is in the stomach in the treated animal from an IP route. There was 12x more food in the stomach 4 hours after treatment and this may have to do with stomach irritation.

Huang asked what would industry do with the panel's findings from the meeting.

Shoaf said EGBE is on the air toxics list and they will set up a risk assessment and then come back 8-9 years later to determine the residual risk. If the risk is large, then they will redo the standards.

Pitot said that if there is no further public comment then the conclusion from the meeting is that we have voted unanimously that the answers are yes to all of the questions. Pitot reminded the panel members to send all of their comment to Brian, and he will collect it and send it back to us altogether.

Shapard said that the comments would be reformatted in the final document. A copy of the discussion will be sent to the chair for verification.

Pitot suggested a telephone conference if there were any outstanding issues.

Shoaf thanked the panel members on behalf of the EPA.

Pitot also thanked the members of the panel for their work and to all of the participants.

There being no further discussion, the meeting was adjourned at 2:40 PM.

Respectively submitted,

J. Chris Corton, Ph.D.
Recorder

**Comments on "An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether" Developed by the National Center for Environmental Assessment of the U.S. Environmental Protection Agency
August 2003 (Interim Final)**

Introduction

Henry C. Pitot:

A review and discussion of the above document, together with associated information was held at the EPA on May 19, 2004. Based on the executive summary of the above document, the EPA felt that prior to making a definitive determination of the classification of EGBE, further information was needed in two areas in relationship to the appearance of statistically significant increases in liver neoplasms, both hemangiosarcoma and carcinoma and forestomach papillomas (benign).

1. Verification of existing PBPK modeling results.
2. Potential relationship of the "genotoxic" effects of BAL *in vitro* on the adverse effects seen in animals following EGBE chronic exposure.

In order to accomplish this objective, the EPA has proposed three specific questions in relation to these points raised with respect to both the liver tumors and the forestomach tumors induced by high levels of EGBE. In these comments each of the three questions will be considered with respect to both of these tissue sites of neoplastic transformation.

Charge to Reviewers

While a consensus is not required on any issue, the panel Chair shall seek a collective opinion from the panel wherever possible.

1. ***EPA's 1999 IRIS assessment and Interim Final position paper place particular focus on hemangiosarcomas of the liver observed in male mice exposed to EGBE because this tumor type was increased over both concurrent and historical controls and because a relatively detailed mode of action involving EGBE has been proposed in the literature. In the position paper, EPA describes a mode of action for this tumor related to iron deposition following hemolysis. However, EPA stated that a definitive determination regarding the role of BAL could not be made and that "additional research (e.g., verification of existing PBPK modeling results and improved genotoxicity assays) would assist the Agency in making a more informed decision concerning the potential for BAL to contribute to the adverse effects seen in animals following EGBE exposure and use of the proposed nonlinear assessment approach." Considering the recent technical submissions (see CD) made in response to EPA's November, 2003 proposed rule:***
 - a. ***Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced liver tumors?***

Xi Haung:

Yes. BAL concentration is measured *in vivo* and is relatively low as compared to EGBE and BAA. However, native Americans and approximately 50% Asian people are deficient in aldehyde dehydrogenases (ALDH). My concern is that these ALDH-deficient people may accumulate high levels of BAL when exposed to EGBE.

ALDH comprises more than nine isoforms in humans (Hsu *et al.*, 1994). Among them, ALDH1, 3, and 9 are in liver cytosol, and ALDH2, 4, 5, and 6 are in liver mitochondria. ALDH 7 and 8 are extrahepatic. ALDH2 is apparently the enzyme that metabolizes shorter chain aliphatic aldehydes such as acetaldehyde. ALDH2*2, as compared to the wild type ALDH2*1, is encoded with a lysine for glutamate

substitution at residue 487 in the enzyme resulting in a loss of enzymatic activity (Crabb *et al.*, 1989). Using human liver from wild type individuals (ALDH2*1/*1), heterozygotes (ALDH2*1/*2) and homozygotes (ALDH2*2/*2), enzymatic activities toward various aldehydes were tested (Kitagawa *et al.*, 2000). Methoxyacetaldehyde (CH₃OCH₂CHO) (MALD) was the aldehyde structurally similar to BAL among the four aldehydes tested. It was shown that the enzymatic activities in mitochondrial fractions were 10% and 0% in hetero (+/-) and homo (-/-), respectively, as compared to wild-type (+/+) of 100%. Using ALDH2 gene knockout mice, similar results were shown.

Do the ALDH deficient people constitute a human subpopulation that is more susceptible to BAL, resulting from EGBE metabolites? This has not been addressed in any literatures cited.

Lisa Kamendulis:

Yes, enough information exists to support an informed decision concerning the BAL metabolite to the formation of EGBE induced liver tumors.

Although a limited amount of data exists showing that BAL produces positive results in tests for genotoxicity, the induction of the effect requires relatively high concentrations of BAL (e.g. 2-fold increases in SCE in human lymphocytes was seen only after 0.5 mM BAL). The additional data supplied with the EPA position paper provides data supporting that the BAL metabolite is not likely to contribute to the formation of EGBE induced liver tumors.

First, the gavage study performed by the Eastman Kodak Health and Environmental Laboratories data measured BAL concentrations in liver following a bolus administration of 600 mg/kg EGBE, a dose higher than that previously shown to induce hemolysis in mice. These studies showed that peak concentrations of 3.26 μM, and 4.16 μM BAL (observed) were produced following 600 mg/kg EGBE in male and female mouse liver, respectively. A comparison of oral and inhalation dose-response simulations for the peak concentrations of BAL in the liver showed that the BAL levels produced following EGBE at 600 mg/kg oral gavage (7.16 μM BAL) would not be achieved even at the theoretical maximal vapor concentration of 1160 ppm (1.63 μM BAL, predicted).

Second, studies examining whether BAL would directly induce DNA damage in murine endothelial cells (target cells) using the Comet assay under alkaline conditions, demonstrated that BAL did not induce damage (single or double strand breaks) to DNA. The potential for BAL to produce DNA damage was examined at concentrations up to the highest noncytotoxic concentration (0.1 mM, 0.5 mM, and 1.0 mM). Comparing the highest concentration used in the DNA damage studies to the PBPK modeling estimates for the determination of peak BAL concentrations, showed that the 1.0 mM concentration used for the evaluation of DNA damage by BAL was much in excess of the value predicted (0.325 μM BAL) following exposure to a carcinogenic level (250 ppm) of EGBE.

Hazel B. Matthews:

Yes. Information is now about as complete as one could expect. It is well established that the metabolic intermediate 2-butoxyacetaldehyde (BAL) is formed in the metabolism of 2-butoxyethanol (EGBE), to its primary metabolite, butoxyacetic acid (BAA). Thus, most of a dose or exposure is metabolized to EGBE is metabolized through BAL to form the terminal metabolite BAA. All available evidence indicates that metabolism of BAL to BAA is a more rapid process than metabolism of EGBE to BAA; therefore, concentrations of BAL are always very low. Even though BAL has been demonstrated to be a weak mutagen when tested in certain systems at relatively high concentrations; there is no evidence that BAL is a mutagen at the concentrations that exist *in vivo*. This observation is supported by the most recent studies of BAL in the Comet Assay that indicate that it does not damage DNA. It is speculated that BAL degrades in the cytoplasm of the cell prior to reaching DNA.

Abraham Nyska:

Issue Hemangiosarcoma in male mice

a. Proposed mechanism of hemangiosarcoma induction in male mice exposed to EGBE:

The NTP Technical Report 484 (NTP, 2000) indicated that the incidences of hemangiosarcoma occurred with positive trend in male mice. The incidence of hemangiosarcoma in males exposed to 250 ppm was

significantly increased relative to the chamber controls and exceeded the range of historical controls (incidence of 0/50, 1/50; 2/49, 4/49, respectively in the control, 62.5, 125 and 250 ppm). The NTP concluded that there was some evidence of carcinogenic activity in the male mouse based on hemangiosarcomas of the liver. In the same study, Kupffer cell pigmentation in the liver was significantly increased over that seen in control animals, in males treated with the two higher doses, and in all female treated groups.

Nyska et al. (2004), retrospectively evaluating the results of 130 two-year carcinogenicity studies conducted in B6C3F1 mice at the NTP, have shown an overall association between liver hemangiosarcoma and Kupffer cell pigmentation to be highly significant ($p < 0.001$) and limited to males. In particular, three compounds (2-butoxyethanol (EGBE), p-nitroaniline, and para-chloroaniline) were associated with a relatively high incidence of Kupffer cell pigmentation consisting of hemosiderin in both sexes; only the male mice developed a relatively low incidence of treatment-related hemangiosarcoma. With a fourth compound (o-nitroanisole), a relatively low incidence (16/50, high-dose males) of chemical-related hemosiderosis was noted, with no associated increase of hemangiosarcoma. It was shown in the same investigation that although a comparable incidence of hemosiderosis was noted in the females, no development of any treatment-related tumor in this organ was noted. The hemosiderosis was related to the hemolytic effect of these compounds. The concluding comment of this investigation was that the data suggest that a significantly increased risk of inducing hepatic hemangiosarcomas in the male B6C3F1 mouse exists in studies with chemicals that cause increased tissue burdens of reactive oxygen species (ROS). The reason for the sex-increased susceptibility for development of hemangiosarcoma is unknown but may be due to a hormone-related, reduced antioxidative defensive capacity through modulation of the activities of antioxidative enzymes.

2-Butoxy-ethanol, via the action of 2-butoxyacetic acid, induced hemolysis in rats and mice (Ghanayem and Sullivan, 1993). 2-Butoxyethanol and 2-butoxyacetic acid, examined in a number of test systems, have not been demonstrated to be mutagenic or directly genotoxic *in vivo* and *in vitro* (Elliot and Ashby, 1997). Similarly, investigators recently reported that BE and 2-butoxyacetic acid failed to induce transformation in the Syrian hamster embryo (SHE) cell transformation model (Park et al., 2002). The mechanisms involved in BE-induced neoplasia are not known; however, indirect or epigenetic mechanisms are apparently involved. Investigating possible mechanisms of hemangiosarcoma induction in mice by BE, Siesky et al. (2002) proposed that the induction of neoplasia by BE occurs indirectly through the induction of oxidative stress, oxidative stress in turn was followed by increased oxidative damage and DNA synthesis, driven by iron deposition in Kupffer cells from red blood cell hemolysis. A biphasic increase in oxidative damage, indicated by increased levels of 8-hydroxydeoxyguanosine and malondialdehyde, and increased DNA synthesis were seen in mouse liver after 7 and 90 days of treatment with BE, whereas no increases were observed in treated rat liver. Both strains were treated with BE, administered daily by gavage, five times per week, at doses of 0, 225, 450, and 900 mg/kg/day (mice) and 0, 225, and 450 mg/kg/day (rats). According to Siesky et al. (2002), the species selectivity (*i.e.*, mice, not rats) for the induction of oxidative stress by BE may be explained in part by differences in antioxidant levels between these rodents. Although BE treatment reduced vitamin E levels in both rat and mouse liver, the basal level was approximately 2.5-fold higher in the rat. The data from these studies suggest that the lack of tumor induction by BE in the rat liver was a result of the substantially greater vitamin E content present over that in the equivalent mouse tissue. Cunningham (2002) in an editorial to Siesky's 2002 paper, concluded that "the oxidative stress mechanism that results in the tumorigenic response in the mouse but not the rat is unlikely to occur in humans. A mouse is not a rat is not a human."

My conclusion from my own experience as well as reviewing the investigations dealing with the EGBE, is that the current available information is adequate to support the mode of action described in the position paper for the EGBE induced formation of hemangio-sarcomas in male mice and the potential relevance of this finding to humans.

Henry C. Pitot:

Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced liver and forestomach tumors?

^a Reviewer combined Question 1a and 2a.

In general, aldehydes, such as formaldehyde and acetaldehyde, are irritants and possess some genotoxic activity, the former also being carcinogenic in at least one tissue, the rat nasal cavity (Swenberg et al., 1983). With increasing carbon length, the primary aldehydes exhibit less to no genotoxic potential. BAL is negative in the standard mutagenic assays. One report (Elliot and Ashby, 1997) indicated that it is clastogenic in Chinese hamster lung (V79) and human lymphocyte cells. However, BAL did not induce DNA single-strand breaks as measured by the Comet Assay at concentrations three orders of magnitude higher than BAL concentrations estimated to occur by PBPK modeling in liver and forestomach (Klaunig and Kamendulis, unpublished results). The study by Siesky et al. (2002) did not show liver DNA adduct formation as indicated in the Interim Final Report (page A2-5, line 4) but did show an increase in 8-hydroxydeoxyguanosine formation in endothelial cells after 7-day exposure to EGBE *in vivo*.

The "genotoxicity" tests on BAL suggest that the chemical has no initiating activity but conceivably might exhibit very weak progressor agent activity because of its weak clastogenic response. However, these tests were carried out *in vitro*, and the concentrations at which chromosomal aberrations occurred in V79 cells in human lymphocytes were reportedly 0.1 to 0.75 mM. The lowest concentration of this is two orders of magnitude higher than the predicted levels of BAL in liver and forestomach (Interim Final Report, pages A2-1A and A1-6). Thus, as stated in the document, it is highly unlikely that BAL has any significant genotoxic effect *in vivo* at the highest concentration studied.

Torka Poet:

Hemangiosarcomas of the liver in male mice

a. Significance of BAL

BAL is unlikely to occur at an appreciable concentration or for a significant length of time. Neither BAL nor butyric acid have been identified following *in vivo* exposures to EGBE. *In vitro*, BAL leads to hemolytic effects only at very high levels, several orders of magnitude higher than would be predicted to occur following oral exposures, as estimated using a PBPK model. BAL might be clastogenic *in vitro*, but only at levels nearing 1 mM (Elliot and Ashby, 1997). A PBPK model that describes the dosimetry of EGBE, BAL, and BAA in several species has been developed and validated (Corley, et al., 2004). Dr. Corley has modified his initial model using estimate from other chemical exposures to calculate liver metabolic rate constants (V_{max}) from values calculated *in vitro* (Green et al., 2002). For similar chemicals, there is a 4-fold difference in hepatic compared to forestomach rates, after adjusting the hepatic V_{max} using this 4-fold correction, the predicted levels of BAL in both GI and liver tissue are within 101% of the measured values reported by Deisinger and Boatman (2004). This model has been used to predict BAL concentrations in the liver following oral and inhalation exposures in mice and in mice with aldehyde dehydrogenase metabolic rates (V_{max}) set at $\frac{1}{2}$ the initial values (R. Corley, personal communication). For inhalation exposures up to the theoretical maximum of 1160 ppm for 6 hr, the prediction BAL liver levels are not going to achieve concentrations above 0.001 mM in these low metabolizing individuals. This predicted maximal concentration is considerably lower than concentrations of BAL shown to be clastogenic (0.2 mM) or hemolytic (0.5 mM: Ghanayem et al., 1989) *in vitro* (Table 1). Therefore, the significance of BAL to hemangiosarcomas is unlikely.

Frank Welsch:

1. Initial assessment of the recent technical submissions made in response to EPA's November, 2003, proposed rule leads this reviewer to conclude that the charge is to make an assessment of those recent technical submissions, as referenced in the cover letter from the American Chemistry Council dated January 20, 2004, with its 4 attachments.

a. New experiments concerning the "Effect of 2-Butoxyacetaldehyde on the Induction of DNA Damage [Comet] in Rodent Endothelial Cells" were conducted by Drs. J.E. Klaunig and L.M. Kamendulis. These investigators designed a study that applied a relevant target cell model [the mouse endothelial cell line, SVEC4- 10], used the suspect and potentially DNA-reactive BAL metabolite and employed the Comet DNA damage assay. That assay method has been applied in Dr. Klaunig's laboratory on previous occasions. The Comet assay outcome with BAL led to the conclusion that BAL failed to induce an increase in DNA damage at subcytolethal concentrations. This outcome allows me to answer the question posed in item 1.a. in the affirmative.

b. Is the current information adequate to support the mode of action described in the position paper for the EGBE induced formation of hemangiosarcomas in male mice and the potential relevance of this finding to humans?

Xi Haung:

Yes. The evidence for BAA causing hemolysis is strong. Although the role for iron in EGBE-induced hemangiosarcoma is weak, this may be due to the insensitivity of Perl's iron staining. Most iron is strongly bound to iron proteins such as ferritin, hemosiderin, transferrin, and therefore, not readily bioavailable for adverse health effects. Iron bound to low molecular weight (LMW) chelators is redox active and, thus, is capable of producing oxidants. It could be of great importance if this fraction of iron has been measured in the animal studies. A newly developed fluorescent calcein method could measure LMW iron in biological fluids of the EGBE-exposed animals (Ali *et al.*, 2003). Protection by various antioxidants, such as vitamin E, supports the oxidative stress mechanism. However, a protection by specific iron chelators, such as deferoxamine, would greatly strengthen the role of iron in the mode of action induced by EGBE. Another aspect that needs to be considered is that iron can be released from transferrin in an acidic environment. BAA is an acid, which may release iron from transferrin.

Lisa Kamendulis:

Yes, the current information is adequate to support the mode of action for EGBE-induced liver hemangiosarcomas in male mice and for determining the potential relevance of this finding to humans.

The step-wise, temporal approach that has been proposed for the events leading to the induction of hemangiosarcomas in the male B6C3F1 mouse is supported by scientific literature and appears adequate to support the mode of action for EGBE induced hemangiosarcomas. Although the key events are agreed upon (induction of hemolysis by BAA, induction of cell proliferation, and clonal expansion), some alternate considerations (also supported by scientific literature) may be involved in the mode of action and are included below.

The induction of hemolysis is a critical step in the proposed mode of action for the induction of hemangioarcomas EGBE. This step has been well documented and is observed in both rats and mice. The induction of hemolysis releases iron from red blood cells and results in a dose related increase in hemosiderin deposition (storage form of iron and an index of RBC/hemoglobin phagocytosis by Kupffer cells) in Kupffer cells in the liver (NTP, 2000; Seisky *et al.*, 2002). Thus, in addition to iron accumulation, the Kupffer cells phagocytize red blood cells damaged by EGBE.

Reactive oxygen species can potentially be derived from two sources: iron overloading in the liver (through Fenton and Haber-Weiss reactions) and/or from Kupffer cell activation. Via either source, oxygen radicals can induce oxidative damage to DNA and lipids as documented in liver following EGBE treatment (Seisky *et al.*, 2002). The activation of Kupffer cells (through phagocytosis of red blood cell hemolytic components

and/or iron in the Kupffer cell), results in the production of cytokines, possibly including vascular endothelial growth factor that may elicit a growth response on endothelial cells. In addition to the production of oxidative DNA damage, reactive oxygen species, whether derived from Kupffer cell activation or other biological processes, can alter gene expression (e.g. MAP kinase/AP-1, and NFκB) resulting in stimulation of cell proliferation and/or inhibition of apoptosis (reviewed in Klaunig and Kamendulis, 2004).

Endothelial cell proliferation is a requirement for the formation of hemangiosarcomas. Previously, the induction of endothelial cell proliferation by 2-butoxyethanol was demonstrated in the male mouse following EGBE at doses that produced hemangiosarcomas in mouse liver (Seisky et al., 2002). The EPA position paper states that the cell proliferation arises from the promotion of preexisting (spontaneously) initiated cells. Whether the increased DNA synthesis and/or oxidative DNA damage results in acquisition of new mutations in endothelial cells or results in a selective clonal expansion of initiated endothelial cells (i.e. functions at the tumor promotion stage of carcinogenesis), has not been established. However, a review of the NTP bioassay results has shown that the mouse liver has a relatively high background of spontaneous endothelial neoplasms in the liver, and lends support to the premise that the induction of liver hemangiosarcomas in the male mouse are the result of tumor promotion mechanisms rather than through production of new mutations.

The EPA position paper highlighted the lack of finding of iron within the target endothelial cell as a potential data gap in the proposed mode of action. However, in this alternate mode of action for EGBE-induced neoplasia, the steps describing activation of Kupffer cells by iron as well as by phagocytosis of EGBE-induced hemolyzed RBC's, and production of reactive oxygen species by iron-mediated reactions as well as through Kupffer cell derived reactions, are included. Thus, the necessity for identifying iron in endothelial cells (presumably to produce reactive oxygen within the target cell?) is lessened. Furthermore, due to the proximity of the endothelial cell to the Kupffer cell within the liver creates an environment in which reactive oxygen species can easily interact and elicit effects on cell populations residing within the liver.

The mode of action for the induction of hemangiosarcomas in mice would be expected to apply to humans (i.e., the key events could occur in humans). However, taking into account kinetic and dynamic factors, the key events in the mode of action are not likely to occur in humans. A critical step in the proposed mode of action for EGBE-induced hemangiosarcomas in male mice is the induction of RBC hemolysis. In vitro studies have demonstrated that levels of BAA (the hemolytic metabolite) need to reach 7.5mM to achieve even early hemolytic changes in human red blood cells (Udden 1994). The existing PBPK models simulate that BAA levels produced at theoretical saturated vapor concentrations would not be in excess of 2mM (Corley et al., 1994).

Hazel B. Matthews:

Yes. BAA has been determined to be the primary hemolytic agent in sensitive species. Hemolysis has been shown to result in accumulation of hemosiderin (iron) in the Kupffer cells of the liver. There is good evidence to indicate that increased high concentrations of iron result in oxidative damage in mouse liver. Thus it is reasonably speculated that chronic oxidative damage resulting from chronic hemolysis induced by BAA accounted the increased incidence of hemangiosarcomas observed in male mouse liver. It has been demonstrated that humans are much less sensitive to the hemolytic effects of BAA. Thus, a similar effect would not be anticipated to occur in humans. Further, with the exception of intentional consumption of EGBE, humans are not likely to encounter this chemical at exposures approaching those to which the mice were exposed. Even then it is highly unlikely that humans would be chronically exposed in such a manner as to induce chronic accumulation of iron in the Kupffer cells of the liver. Therefore, it is reasonable to assume the increased incidence of hemangiosarcomas observed in the male mouse liver are not of relevance to humans.

^b Reviewer combined Question 1b and 2b

Abraham Nyska:

b. Issue of suggested nonlinear cancer assessment approach to be applied for the male mouse liver tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hemolytic effects in humans would prevent the formation of liver tumors in humans)?

Metabolic activation of EGBE to form butoxyacetic acid (BAA) is a prerequisite for the development of hematotoxicity (Ghanayem 1996). Recently investigators demonstrated that human erythrocytes require exposure to a 100-fold greater concentration of BAA to develop changes involving red cell deformability, osmotic fragility, and sodium content similar to those observed in rat erythrocytes (Udden 2002). It was shown that human erythrocytes are sensitive to the EGBE, and that exposure to extremely high doses induce hemolytic effects (Burkhart and Donovan, 1998). However, thorough investigations by Udden (2002) and Ghanayem and Sullivan (1993) demonstrated that the erythrocytes of mice and rats are sensitive to the hemolytic effect of the BAA while erythrocytes of human are relatively insensitive.

Previous discussion presented data which was supportive of the suggested promotion of iron deposits (hemosiderin) on the induction of relatively low incidence of liver hemangiosarcoma in male mice. The data supports the suggested mode of action of EGBE, inducing liver hemangiosarcoma in male mice, and therefore, it can be expected that exposure of human to relatively low doses which will not induce hemolytic effect, are also expected not to pose risk of hemangiosarcoma induction, similarly to the mechanism suggested in mice. **It is, therefore, my recommendation that the nonlinear cancer assessment approach will be applied for the male mouse liver tumors observed following EGBE exposure.**

Henry C. Pitot:

Is the current information adequate to support the mode of action described in the position paper for the EGBE induced formation of hepatic hemangiosarcomas and forestomach tumors in male and female mice respectively and the potential relevance of this finding to humans? ²

The data presented in the Interim Final Report appears quite adequate to support the mode of action described for the formation of hemangiosarcomas. Despite the considerable evidence for the oxidative effect of high levels of iron that occur in cells, one of the most telling arguments is the fact that at least three other unrelated chemicals that induce high levels of hemosiderin deposition in the liver also induce hemangiosarcomas (Table A2-1). Hemosiderin is the term utilized by pathologists to indicate iron deposition in cells in general. Where it has been looked at chemically, this almost always indicates the association of iron with protein, many times in one of the forms of ferritin. This is especially true in hepatocytes where apoferritin synthesis is regulated by iron. This is also true in other cells, especially of the RE system (Knutson and Wessling-Resnick, 2003). It is difficult for this reviewer to believe that the iron deposition in the livers of these animals did not occur in Kupffer and endothelial cells, although the report suggests (footnote Figure A2-1) that there was not explicit mention of iron deposition in these cell types. If this point becomes important, it is readily checked by a reexamination of the histology and if there is any question, ultrastructural studies may be carried out, even on formalin-fixed tissues. The argument for hepatocellular carcinomas that were noted, really only at the highest dose, may similarly be applied since *p*-chloroaniline hydrochloride, which also produced hemosiderin deposition at the high dose induced hepatocellular carcinomas in a similar manner. The argument, however, is that a trend is present and when adenomas and carcinomas are considered together, there is no significant change. Since it has usually been the policy of EPA and other federal agencies not to distinguish between benign and malignant neoplasms, particularly in mouse liver, it would appear that the carcinoma change is not significant.

However, one could make an argument that if EGBE or its BAL metabolite are clastogenic, the higher levels of carcinomas may be a result of the effect of such clastogenic properties (Pitot, 2002). The development of squamous cell papillomas in the forestomach of female mice exposed to EGBE is most readily explained on the basis of a promoting action of the extensive cell proliferation/hyperplasia in the squamous cells of the forestomach of animals administered EGBE. As noted from the Interim Final Report, there is ample evidence that the material, when administered, remains within the stomach for considerable periods as an irritant. Such changes were seen in all mice and all rats but only female mice showed the presence of benign lesions. The presence of a single carcinoma merely indicates the spontaneous transition from cells in the stage of promotion (papilloma) to those in the stage of progression

(carcinoma). The fact that there was regression of the "precancerous forestomach lesions" in animals treated with EA for 6 months and allowed 2 or 15 months of recovery with the development of no forestomach neoplasm were seen. Extending initial treatment for another 6 months did result in the presence of neoplasms following a 2-month "recovery", indicating that some of these foci or lesions had already developed into the stage of progression (Pitot, 2002). Although EA, an unsaturated aldehyde, is not a metabolite of EGBE, it is an analog of BAL and a much more potent carcinogen (Gold et al., 1993).

The interesting susceptibility of the forestomach of female mice as opposed to male mice and rats is not unusual as evidenced by the susceptibility of male mice to hepatomas in contrast to female mice following administration of a variety of carcinogens.

Thus, if further studies were to be done in this area, they may be oriented towards the possible role of female sex hormones in the neoplastic response to the chronic inflammation and cell proliferation in the forestomach seen in females. All of these *in vivo* data, coupled with the fact that there is no substantial *in vivo* data demonstrating the genotoxicity of EGBE or BAL as noted in the Interim Final Report, argues that the neoplasms seen in the forestomach of the female mouse are due to a promoting action of the prolonged presence of EGBE in inducing the cell proliferation in the forestomach and subsequent promotion of spontaneously initiated cells in the squamous epithelium. The effect of sex hormones on this phenomenon would be of interest to study, although results obtained would be of more academic interest than potentially useful in risk estimation.

Torka Poet:

b.mode of action

There is considerable evidence that the mode of action for the development of hemangiosarcomas in the liver is related to hemolysis. The precise mechanism that links hemolysis to the hemangiosarcomas is less well explained. However, since human cells are resistant to the primary hemolysis step the relevance of these modes of action to humans is questionable. Thus, regardless of mechanism leading from hemolysis to liver effects, the relevance to humans is unlikely. Also, the overall weight of the evidence is strong enough to substantiate areas with less data. The primary mode of action, hemolysis, has a compelling amount of evidence to substantiate it regardless of the subsequent steps leading to the hemangiosarcomas.

Frank Welsch:

b. Attachment 2 [Aug., 2003] of the position paper provides a carefully assembled overview and analysis of the state of scientific knowledge about the EGBE-induced formation of hemangiosarcomas in male mice. Further, that document addresses at length the issue of relevance of mouse liver hemangiosarcomas and hepatocellular carcinomas to humans. In my reading and interpretation of the scientific facts reviewed in Attachment 2, item 1. b., may be answered in the affirmative.

c. Does the available information support a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hemolytic effects in humans would prevent the formation of liver tumors in humans)?

Xi Haung:

Yes. The available information supports a nonlinear cancer assessment approach. However, prevention of hemolytic effects in human may not be enough to prevent liver cancer in humans. BAL might cause DNA-protein crosslinking. BAA may cause iron release from transferrin. Considering that iron stored and transported by transferrin is in micromolar range, this mechanism of EGBE-induced oxidative damage may not be negligible.

Lisa Kamendulis:

Does the available information support a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure, ~~(i.e., and therefore is it reasonable to expect that the prevention of hemolytic effects in humans would prevent preclude the formation of liver tumors in humans)?~~

Yes, the available data supports a nonlinear cancer assessment approach for the induction of liver tumors in male mice following EGBE exposure. Furthermore, it is reasonable to expect that a lack of hemolytic effects in humans would preclude the formation of liver tumors in humans.

A potential alternative mode of action involving direct DNA reactivity by BAL was previously postulated. However, as outlined in question 1a above, the contribution of BAL to the induction of liver tumors in male mice is not likely a contributing factor in the observed neoplasia, based on pharmacokinetic factors and data demonstrating a lack of induction of DNA damage in endothelial cells by BAL.

As indicated in question 1b above, the mode of action for the induction of hemangiosarcomas in mice would be expected to apply to humans (i.e., the key events could occur in humans). However, taking into account kinetic and dynamic factors, the key events in the mode of action is not likely to occur in humans.

Hazel B. Matthews:

Yes. The increased incidence of hemangiosarcomas was observed only at the highest dose and only in male mice, thus, this appears to be a dose, sex and species dependent effect. The observation of dose and species effects might be explained by the facts that rats were exposed to only half the highest dose as mice and mice inhale a greater volume of air per gram body weight than. Also, rats are historically known to be less sensitive to the development of liver tumors than mice. The sex dependent effects might be explained by the fact that male mouse liver is well known to be more sensitive to the development of tumors than female mouse liver. Thus exposure to a similar concentration of EGBE may not have been quite sufficient to induce a similar increased incidence in female mice. The relevance of these observations to humans is discussed in (b) above.

Abraham Nyska:

c. It was convincingly demonstrated that the active metabolite (e.g. butoxyacetic acid) is the major metabolite of the EGBE, which is responsible for the hematologic toxicity of this compound.

^c Reviewer changed wording of the question.

Henry C. Pitot:

Does the available information support a nonlinear cancer assessment approach for the development of male mouse liver tumors and for the development of forestomach tumors in the female mouse observed following EGBE exposure?^d

The effect of BAA on inducing hemolysis in rats and mice is considerably greater than that in humans (page A2-19). Thus, unless humans were exposed to extremely high doses for extended periods (years), there is really no obvious relationship between the findings seen in rats and mice and those in humans. The only possible group that might be affected (to this reviewer's knowledge) might be the hemochromatosis heterozygote that comprises some 12% of the human population (Barton and Bertoli, 1996). Smaller amounts of hemolysis in these individuals could lead, over extended periods, to some chronic iron deposition in hepatocytes, but it would seem unlikely that even such individuals would have any problem. Thus, based on the animal data and the enforcement of the low dose ranges for EGBE proposed in the Interim Final Report, there should be no risk to the human, and for the cancer assessment it is appropriate to use a nonlinear approach.

In the case of mouse forestomach tumors in females, the available rodent data argue strongly for a nonlinear cancer assessment approach. It is highly unlikely that any of the forestomach data applies to the human for several reasons. Some of these are indicated in the Interim Final Report. The normal human has no structure comparable to the forestomach in the rodent and even more important is that esophageal and gastric emptying occur relatively rapidly within the human (a matter of minutes to a few hours) unlike the mouse and rat under the conditions of the assay. Secondly, while chronic irritation at the gastroesophageal junction induced by acid reflux is increasing in incidence (Voutilainen et al., 1999) as is Barrett's Esophagus. The causes of these conditions are virtually identical to that seen in the rodent forestomach, i.e. chronic inflammation and induced cell proliferation. For individuals so affected, any possible contribution by exposure to the small levels of EGBE would be virtually nonexistent, in this reviewer's opinion. Thus, a nonlinear cancer assessment approach is certainly appropriate, but is, in this reviewer's opinion, essentially academic, having virtually no application to human gastroesophageal neoplastic lesions.

Torka Poet:

c. non-linear cancer assessment approach

The evidence outlined above all leads to the conclusion that the effects following EGBE exposures in mice are secondary to hemolytic effects. Therefore, a non-linear risk assessment is the correct approach.

Frank Welsch:

c. Attachment 2 [Aug., 2003] also describes in detail the scientific data that support a nonlinear mode of action for the end-point liver tumors in male mice. The remaining uncertainty expressed in that document at the time of its completion relates to the possibility that BAL, as an EGBE metabolite, might have the potential to interact directly with DNA. In addition, there was concern about more validation of PBPK simulations that had cast doubt on the high BAL concentrations used in *in vitro* assays and their relevance to those achievable under much more realistic *in vivo* exposure conditions. Both topics have been critically addressed by new experimental studies. Therefore, it is now possible to conclude that the non-linear cancer risk assessment approach is reasonable and backed by solid scientific data and arguments.

^d Reviewer combined Question 1c and 2 c

2. ***NTP (2000) also identified forestomach tumors in female mice following EGBE exposure. In its position paper, EPA describes a mode of action for this tumor related to retention in the forestomach, metabolism to BAA, irritation and cell proliferation. However, EPA again stated that a definitive determination regarding the role of BAL could not be made and that “additional research (e.g., verification of existing PBPK modeling results and improved genotoxicity assays) would assist the Agency in making a more informed decision concerning the potential for BAL to contribute to the adverse effects seen in animals following EGBE exposure and use of the proposed nonlinear assessment approach.” Considering the recent technical submissions (see above) made in response to EPA’s November, 2003 proposed rule:***
- a. ***Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced forestomach tumors?***

Xi Haung:

Yes. Levels of BAL in ALDH-deficient people may be a concern (see point 1a).

Lisa Kamendulis:

Does enough information now exist to support an informed decision concerning the significance of the BAL metabolite genotoxicity to the formation of EGBE induced forestomach tumors? Yes, enough information exists to support an informed decision concerning the BAL metabolite to the formation of EGBE induced forestomach tumors.^e

As outlined in question 1a above, the additional data supplied with the EPA position paper provides adequate data to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced forestomach tumors.

The gavage study performed by the Eastman Kodak Health and Environmental Laboratories data measured BAL concentrations in GI following a bolus administration of 600 mg/kg EGBE. These studies showed that peak concentrations of 18.11 μ M, and 32.99 μ M BAL (observed) were produced following 600 mg/kg EGBE in male and female mouse GI, respectively. As was shown for BAL concentrations in the liver, using a comparison of oral and inhalation dose-response simulations for the peak concentrations of BAL in the GI, the BAL levels produced following EGBE at 600 mg/kg oral gavage (19.93 μ M BAL) fall far short of those that would be achieved at the theoretical maximal vapor concentration of 1160 ppm (3.82 μ M BAL).

Hazel B. Matthews:

No. It has not been determined which chemical accounts for the observed increased irritation that most probably accounts for the increased incidence of forestomach tumors. The rodent forestomach, both rats and mice, has, in numerous previous NTP studies, been observed to develop an increased incidence of tumors when subjected to chronic irritation resulting from administration of both genotoxic and non-genotoxic chemicals. Thus, in the present case it can not be conclusively determined if the parent chemical, EGBE, or one of its major metabolites, BAL and BAA, accounted for the chronic irritation that resulted in the observed increased incidence of forestomach tumors. As discussed above, results of the Comet assay indicate that the more reactive metabolite, BAL, which is formed in the cytoplasm most probably, does not survive in the cell sufficiently long to reach the DNA in the nucleus. Thus, available information indicates that BAL does not result in genetic alteration that leads to an increased incidence of forestomach tumors. However, it has not been determined that this relatively reactive metabolite does not account, at least in part, for the chronic irritation that results in an increased incidence of tumors. Further, since it is not possible to administer EGBE or BAL to intact animals without their rapid metabolism to BAA conclusive determination of the irritating chemical is probably not feasible.

Abraham Nyska:

Issue squamous cell pailloma and squamous cell carcinoma in female mice

^e Reviewer changed wording of the question.

a. Proposed mechanism of forestomach tumor induction in female mice exposed to EGBE: The NTP Technical Report 484 (NTP, 2000) indicated that the incidences of squamous cell papilloma and squamous cell carcinoma (combined) occurred with a positive trend in females, and the incidences in females exposed to 250 ppm were significantly increased relative to the chamber controls. The incidences exceeded the ranges for historical controls. In male mice exposed to 125 or 250 ppm, the incidences of squamous cell papilloma also exceeded the range of historical controls. Also, the incidences of ulcer in the forestomach were significantly increased relative to the chamber controls in males exposed to 125 ppm and in all exposed groups of females. The incidences of squamous epithelial hyperplasia, usually focal, were significantly increased in all exposed groups of males and females. In the preliminary 14-week study, epithelial hyperplasia and inflammation of the muscularis or serosa of the forestomach occurred in females exposed to 125 ppm or greater.

Poet et al. (2003) performed several experiments in order to demonstrate the particular sensitivity of the mouse forestomach to EGBE, administering this compound by various routes. Oral administration of undiluted BE was shown to cause irritation and a compensatory proliferative response in the mouse forestomach, confirming that direct contact between the forestomach and BE, which can occur via grooming of BE condensed on the fur during inhalation exposures, can cause irritation. In addition, parenteral administration of this compound (ip and sc injection) also resulted in forestomach lesions, indicating that there may be sources other than grooming for EGBE- or BAA-induced forestomach irritation. In the pharmacokinetic study, EGBE and, to a lesser extent, BAA was eliminated more slowly from the forestomach tissue of mice than from blood or other tissues, following either oral gavage or ip injection. The forestomach was the only tissue with detectable levels of EGBE at 24 h. EGBE and BAA were both excreted in the saliva and were present in stomach contents for a prolonged period of time following these routes of exposure, which may further contribute to forestomach tissue dosimetry. These investigations demonstrated that there appear to be multiple mechanisms behind the increased levels of BE and BAA in the forestomach tissue of mice, which together can contribute to a prolonged contact irritation, compensatory hyperplasia, and tumorigenicity in mice. Administration of 14 C-labeled EGBE, using the IV and inhalation exposure, demonstrated particularly high levels of the label in the forestomach and much less in other analyzed organs (Green et al., 2002). It was also shown that the forestomach of mice contain higher activity of alcohol dehydrogenase than rat, and therefore the metabolic activation of the toxic metabolite is higher in mice than rats. The forestomach of mice are also more prone than rats to develop spontaneous forestomach squamous cell tumors, indicated by the higher incidences of these tumors in the historical data of mice than rats.

There are numerous examples of nongenotoxic carcinogens, such as the EGBE, inducing relatively low incidence of forestomach tumors following 2-years exposure to this compound. In the case of EGBE, it is suggested that the forestomach cytotoxicity results from sustained irritation of the squamous epithelium by this compound or its metabolite, leading to inflammation, ulceration, and regenerative hyperplasia. The female mice had particularly severe irritation in the forestomach, expressed in relatively high incidence of ulcers, inflammation and hyperplasia, seen at low incidence or completely absent in the males treated with the same dose, which is of particular support for the non-genotoxic mode of action in the case of the female forestomach carcinogenesis.

The time of contact of the food containing the irritating compound has major importance for the promotion of forestomach tumors. The forestomach is unique structure in rodents that does not exist in human, even though the human esophagus is lined by a comparable squamous epithelium. The irritation and hyperplasia of the forestomach are essential stages for the development of tumors by EGBE, and exposing human to doses below the threshold of irritation is expected to pose risk of tumorigenicity.

My conclusions from my own experience as well as reviewing the investigations dealing with the EGBE, is that the current available information is adequate to support the mode of action described in the position paper for the EGBE induced formation of forestomach tumors in female mice and the potential relevance of this finding to humans.

Henry C. Pitot:

See 1a.

Torka Poet:**1. Forestomach tumors in female mice****a. Significance of BAL**

The weight of evidence strongly suggests that BAA is the primary irritant that leads to epithelial proliferation and the forestomach lesions seen in female mice. BAL is likely to be transient and unlikely to occur at an appreciable concentration or for a significant length of time. The irritant effects following EGBE exposures require time to develop. The model of Corley et al (personal communication) has been used to predict BAL concentrations in the GI tract following oral and inhalation exposures in mice and in mice with aldehyde dehydrogenase metabolic rates (V_{max}) set at $\frac{1}{2}$ the initial values. Following very high oral doses of EGBE, the model predicts high concentrations of BAL may be achieved. Only after oral doses of 300 mg/kg are BAL levels comparable to those found to have an effect in vitro. For inhalation exposures up to the theoretical maximum of 1160 ppm for 6 hr, the prediction BAL liver levels are not going to achieve concentrations above 0.01 mM in these low metabolizing individuals (Table 1). This predicted maximal concentration is considerably lower than concentrations of BAL shown to be clastogenic (0.2 mM) or hemolytic (0.5 mM: Ghanayem et al., 1989) in vitro (Table 1). While BAL has only been detected at very low levels in blood following high oral EGBE doses, BAA has been detected over time and at relatively high levels in the forestomach of female mice following in vivo exposures to EGBE through multiple routes. Data indicate that BAA is sequestered in the outer layers of forestomach tissue. Exposures to BAA demonstrate that it is more a potent forestomach irritant than EGBE.

Table 1. Dose-response simulations of the peak tissue concentrations (Cmax) of butoxyacetaldehyde (BAL) in female mice following either oral gavage or 6-hr inhalation exposures. To simulate a heterozygous population with lower aldehyde dehydrogenase activity, simulations with ½ the Vmax rate are also shown.

		Cmax BAL Liver	Cmax BAL Liver @ ½ Vmax	Cmax BAL GI Tract	Cmax BAL GI Tract @ ½ Vmax
Route or Exposure (ppm)	Dose (mg/kg)	(µM)	(µM)	(µM)	(µM)
Oral	1	0.002	0.004	0.068	0.136
	10	0.021	0.043	0.686	1.399
	25	0.056	0.112	1.755	3.686
	50	0.123	0.247	3.644	8.090
	100	0.305	0.615	7.85	19.95
	150	0.584	1.187	12.57	38.22
	300	2.241	4.773	25.07	160.5
	500	4.211	9.502	31.61	419.2
	600	4.586	10.47	32.99	525.3
	900	4.991	11.53	34.96	725.6
Inhalation	1	0.000	0.001	0.003	0.006
	5	0.002	0.003	0.015	0.030
	10	0.003	0.006	0.030	0.061
	25	0.008	0.016	0.076	0.153
	50	0.016	0.032	0.153	0.307
	63	0.020	0.040	0.193	0.388
	100	0.032	0.064	0.307	0.619
	125	0.040	0.080	0.384	0.776
	150	0.048	0.096	0.462	0.935
	200	0.064	0.129	0.618	1.257
	250	0.081	0.162	0.775	1.583
	500	0.164	0.329	1.576	3.292
	750	0.249	0.502	2.404	5.141
	950	0.320	0.645	3.086	6.732
	1160	0.395	0.799	3.820	8.519

Frank Welsch:

2. In the August 2003 position paper, the EPA seems to concur with the scientific evidence that has emerged over many years as a result of the accumulated data from many research studies. EPA recognizes the proposed mode of action for forestomach tumors in female mice as plausible. However, EPA remained concerned about the role of BAL, validation 2 of existing PBPK models and improved genotoxicity data that would better define the possible role of BAL.

The recent technical submissions provided by the ACC on January 26, 2004, have been responsive to the concerns raised in EPA's November 2003 proposed rule.

a. The combined new scientific data from the BAL evaluation for potential DNA interactions in the Comet assay, the detailed pharmacokinetic studies conducted in the Eastman Kodak Laboratories and the expanded PBPK validations provide enough new information to allow an informed decision supporting the criteria spelled out in item 2.a.

b. Is the current information adequate to support the mode of action described in the position paper for the EGBE induced formation of forestomach tumors in female mice and the potential relevance of this finding to humans?

Xi Haung:

Yes. If humans have no comparable organ to that of rodents and the mode of action in rodents is due to deposition and retention of EGBE and BAA, the risk for humans to develop GI tumors may be minimal. One question is why forestomach tumors only occur in female mice. Besides all plausible explanations for this observation, specific enzymes related to estrogen metabolism may contribute to this forestomach tumor development in female mice.

Lisa Kamendulis:

Yes, the current information is adequate to support the mode of action for EGBE-induced forestomach tumors in female mice and for determining the potential relevance of this finding to humans.

The step-wise, temporal approach that has been proposed for the events leading to the induction of forestomach tumors in the female B6C3F1 mouse are supported by scientific literature and appear adequate to support the mode of action for EGBE induced forestomach tumors.

The EPA position paper states that the cell proliferation arises from the promotion of preexisting (spontaneously) initiated cells. Whether the increased DNA synthesis results in acquisition of new mutations or results in a selective clonal expansion of initiated cells (i.e. functions at the tumor promotion stage of carcinogenesis), has not been established.

The mode of action for the induction of forestomach tumors in mice would be expected to apply to humans (i.e., the key events could occur in humans). However, taking into account kinetic and dynamic factors, the key events in the mode of action is not likely to occur in humans. Due to pharmacokinetic differences for EGBE between humans compared with rodents, and dynamic differences of the rodent forestomach (a lack of an analogous anatomical organ in humans), mucosal protection/buffering system in the glandular stomach of the human, and lack of grooming in humans, the probability for irritation and the sequelae of events leading to forestomach neoplasia is not likely to occur in humans.

Hazel B. Matthews:

Current information is good, but not as good as it might be. There is a great deal we do know about the formation of increased incidences of tumors in response to chronic irritation. This is but one example of many. To this end I do not think additional PBPK modeling would significantly enhance our understanding of the mechanisms of tumorigenesis involved. However, more careful studies of the fate of EGBE in forestomach might prove helpful in determining just how much of each of the three chemicals, EGBE, BAL and BAA, are present in forestomach cells, how long they persist and why EGBE in drinking water did not

induce similar lesions/tumors. These studies would probably still not conclusively resolve the question as to which of the three chemicals account for most of the chronic irritation that lead to an increased incidence of tumors.

Abraham Nyska:

b. It was convincingly demonstrated that the active metabolite (e.g. butoxyacetic acid) is the major metabolite of the EGBE, which is responsible for the forestomach toxicity of this compound.

Henry C. Pitot:

See 1b

Torka Poet:

b. mode of action

The lack of positive effects of EGBE, BAA, or BAL on in vitro assays strongly suggest a non-genotoxic mechanism. Also, the progression of lesions observed and the sequestration of BAA in forestomach tissue indicates an irritant effect while chemical is present. Areas in which evidence for a mode of action are less robust are also associated with more questionable relevance to humans. The lack of effect observed following drinking water studies may indicate a buffering of this irritating effect from the water vehicle or, more likely, a dose-rate effect. In addition, neither EGBE nor its major metabolite binds to stomach macromolecules.

Frank Welsch:

b. The accumulated scientific data now available support the mode of action described in EPA's August 2003 position paper. The relevant data are further elaborated upon by the cover letter of ACC dated January 20, 2004.

Attachment 1 [August 2003] of EPA's position paper deals with the end-point "*Forestsomach Tumors in Female Mice*" in a comprehensive state-of-the-science manner. The new data generated by Deisinger and Boatman on *in vivo* metabolism and kinetics of EGBE pay specific attention to the forestomach issue. The data compare kinetics in this *rodent specific* tissue of the esophageal-gastric canal to liver and blood kinetics of EGBE, BAL and BAA. The review article provided [Boatman et al., Journal of Toxicology and Environmental Health, 2004, in press] in the attachments of the ACC letter of January 20, 2004 reflects the combined efforts of reputable senior investigators in academia, a contract research organization and industry. That review included the latest PBPK model expansions by Corley and associates of PNNW Laboratories, and the pharmacokinetic validations of the expanded PBPK modeling efforts. Furthermore, specific analytical measurements were made regarding the response of female mouse forestomach irritation to various high doses of EGBE given by multiple routes of administration. The results revealed that the systemic distribution of EGBE and/or its metabolites did induce forestomach lesions, which were apparently independent of direct contact with EGBE. The review article by Boatman et al. [2004, in press] included a comprehensive assessment of forestomach tumors in rodents and their relevance for human carcinogenicity. I concur with the overall conclusions that the weight of the scientific evidence for EGBE and its metabolites indicates that these tumors in female mice are unlikely to be relevant to human risk assessment. Those tumors were caused by irritation in an organ for which no structurally or functionally similar counterpart exists in man.

c. Does the available information support a nonlinear cancer assessment approach for the female mouse forestomach tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hyperplastic effects in humans would prevent the formation of gastrointestinal tumors in humans)?

Henry C. Pitot:

See 1c

Xi Haung:

Yes. The available information supports a non-linear cancer assessment approach. However, what metabolites of EGBE causing hyperplasia are not known. Prevention of hyperplastic effects in humans may not be enough for the prevention of GI tumors.

Lisa Kamendulis:

Does the available information support a nonlinear cancer assessment approach for the female mouse forestomach ~~liver~~ tumors observed following EGBE exposure, ~~(i.e., and therefore is it reasonable to expect that the prevention a lack of hyperplastic effects in the region of the gastroesophageal junction in humans would prevent preclude the formation of gastrointestinal tumors in humans)?~~

Yes, the available data supports a nonlinear cancer assessment approach for female forestomach tumors following EGBE exposure. Furthermore, it is reasonable to expect that a lack of hyperplastic effects in the region of the gastroesophageal junction would preclude the formation of gastrointestinal tumors in humans.

A potential alternative mode of action involving direct DNA reactivity by BAL was previously postulated. However, as the comments outlined under question 2a above indicate, the contribution of BAL to the induction of forestomach tumors in female mice is not likely to contribute to the observed neoplasia based on pharmacokinetic factors.

As noted in the response to question 2b above, the mode of action for the induction of forestomach tumors in mice would be expected to apply to humans (i.e., the key events could occur in humans). However, taking into account kinetic and dynamic factors, the key events in the mode of action is not likely to occur in humans.

Hazel B. Matthews:

Matthews: Does the available information support a nonlinear cancer assessment approach for the female mouse ~~liver~~ forestomach tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hyperplastic effects in humans would prevent the formation of gastrointestinal tumors in humans)?[†]

Yes. The rodent forestomach has, in numerous previous NTP studies, been determined to develop and increased incidence of tumors when subjected to chronic irritation. In virtually all cases the same chemicals did not induce an increased incidence of forestomach tumors when administered at doses that did not result in chronic irritation and in many if not most cases did not induce tumors distant from the forestomach. In the present case, though it can not be conclusively determined if the parent chemical, EGBE, or one of its major metabolites, BAL and BAA, accounted for the chronic irritation, there is a clear association between chronic irritation and an increased incidence of forestomach tumors. As discussed above, in the absence of intentional consumption, humans will not encounter similar exposures to EGBE and even then the exposures would be acute rather than chronic. Thus, it would appear that the forestomach tumors observed in female mice are not relevant to humans.

[†] Reviewer changed wording of the question.

⁹ Reviewer changed wording of the question.

Abraham Nyska:

c. Issue of suggested nonlinear cancer assessment approach to be applied for the female mouse forestomach tumors observed following EGBE exposure (i.e., is it reasonable to expect that the prevention of hyperplastic effects in humans would prevent the formation of liver tumors in humans)? Previous discussion presented data which was supportive of the suggested promotion of forestomach irritation and hyperplasia on the induction of relatively low incidence of forestomach squamous cell tumors in female mice. The data supports the suggested mode of action of EGBE, inducing forestomach tumors in female mice, and therefore, it can be expected that exposure of human to relatively low doses which will not induce hyperplastic effect, are also expected not to pose risk of forestomach induction, similarly to the mechanism suggested in mice. It is therefore my recommendation that the nonlinear cancer assessment approach will be applied for the female mouse forestomach tumors observed following EGBE exposure.

Torka Poet:

c. non-linear cancer assessment approach

The evidence outlined above all leads to the conclusion that the potential forestomach lesions following EGBE exposures in mice are secondary to irritant effects. Therefore, a non-linear risk assessment is the correct approach.

Frank Welsch:

c. The question posed to the reviewers in this item is confusing as regards the mouse gender specific target organ carcinogenicity. The tumor bioassay results in B6C3F1 mice revealed two tumor types leading to the classification of "some evidence" of carcinogenicity: Liver hemangiosarcomas in male mice, an end3 point that was previously addressed in the charge to reviewers item 1b and 1c. [See above comments.]

The second end-point of tumorigenicity in the EGBE bioassay was forestomach tumors in female mice. Item 2.b. in the charge to reviewers raises questions about those forestomach tumors. Item 2.c. seems to attach the wrong gender [female] to a target organ that has already been discussed. Hyperplastic effects in the forestomach of female mice are the hallmark of the response of that tissue to EGBE and metabolites, most likely BAA.

The liver response is entirely different and does not involve a hyperplastic tissue response. Thus, in my opinion, the issue to be addressed deals with the question as to whether the non-linear cancer assessment approach is applicable to **female forestomach tumors**.

If the scientific evidence from the extensive animal studies is accepted as regards cause and effect association of EGBE-BAA exposure with local forestomach irritation leading to hyperplastic responses, then the physiological differences in esophageal-gastric anatomy and the residence time of food-stuffs between mice and man indicate that the female mouse forestomach effects would be highly unlikely to occur in man.

In addition to preparing written comments which address the issues above, feel free to provide any additional comments or recommendations you feel are important to this assessment. If your suggestions include references to published material, please provide a photocopy of the cited material. Feel free to make legible notations in the page margins and return those annotated pages with your written comments. If your comments are limited to particular sections of these documents or to particular issues, please indicate clearly the limitations of your review.

Additional Comments and References

Xi Haung:

Native Americans and Asians are known to be deficient in aldehyde dehydrogenases. It is possible that this population may have a high accumulation of BAL in the body when exposed to EGBE. If BAL is the active metabolite, this particular population may be more susceptible to the adverse health effects of EGBE. Since ALDH2 knockout mice are similar to human ALDH2*2/*1 and ALDH2*2/*2 subjects, it may be advisable to measure BAL in the heterozygote and homozygote mice after EGBE administration. This issue should be addressed by EPA.

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Lisa Kamendulis:

The language of the questions in the charges to reviewers has been changed to reflect the consensus opinion of the panel in discussions held on May 19, 2004.

No additional comments need to be addressed.

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Hazel B. Matthews:

- A. I did not see a description of how mice were housed, i.e., group housed and treated and/or held separately. Male mice are often held separately because they fight when group housed, but this is usually not the case with females. This information could be significant to interpretation of available information, as group housing of females and individual housing of males would permit an opportunity for the females to groom one another and thus receive a higher dose of EGBE to the forestomach.

- B. It is possible that an increased incidence of forestomach tumors were observed in the inhalation studies, but not in drinking water studies because the drinking water studies did not permit consumption of neat EGBE as a result of condensation on the airways and/or grooming. Also, the fact that EGBE consumed in drinking water studies was diluted in water could account of the different results because previous work has demonstrated a positive correlation between the concentration of a chemical and its irritation of forestomach tissue. And finally, I do not think water, and thus EGBE consumed in drinking water, would be retained in the forestomach, as is the case with food and EGBE consumed through inhalation and/or grooming. Thus, the inhalation study may have achieved both more concentrated and more prolonged exposure of the forestomach to EGBE than did the drinking water study.
- C. Overall I think the EPA staff responsible for this evaluation have done an outstanding job of compiling, analyzing and summarizing available data for EGBE.

Abraham Nyska:

References:

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Henry C. Pitot:

Conclusions

EGBE is an important industrial solvent. Chronic bioassays of this chemical in mice and rats demonstrated the induction of hepatic angiosarcomas in male mice and a low incidence of benign forestomach neoplasms in the female mouse. Mechanisms for the development of these two lesions in the mouse are quite satisfactorily documented, and the absence of significant *in vivo* genotoxic toxicity as well as the unlikely application of *in vitro* studies exhibiting clastogenicity of the intermediate metabolite, BAL, argues strongly that a nonlinear cancer assessment approach should be utilized for any possible application of these data to risk estimations in the human. There appears to be virtually no human risk associated with the mechanisms involved in the development of these two types of neoplasms in the mouse, and thus the RfD and RfC for EGBE appear to be very appropriate as levels below which exposure of humans to EGBE even for chronic periods would pose any risk of carcinogenic or non-carcinogenic effects.

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Frank Welsch:

Having had many years of research experience with ethylene glycol monomethylether, EGME, I can appreciate very much the PBPK modeling and validation work conducted by Dr. Corley and his associates. EGME is the most potent reproductive and developmental toxicant among the monoalkyl substituted ethylene glycol ethers. Our team collected extensive data on route and mode of administration. The studies involved substantial efforts regarding toxicokinetics and toxicodynamics. Furthermore, PBPK models and their validation, including interspecies extrapolations, was a significant part of that research effort.

The debate involving the role of the aldehyde intermediate, 2-methoxyacetaldehyde [MALD], was never as intense as the one surrounding BAL. Claims had been made, based on *in vitro* studies, with high MALD exposure concentrations, that MALD could cause mutations, sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells as well as chromosomal damage in human lymphocytes. However, all of those effects were achieved under artificial exposure conditions with no relevance to the *in vivo* situation.

**Conclusions of Review Meeting on the EPA 2003 Interim Final Position Paper entitled “An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether”,
Held at the Environmental Protection Agency, Research Triangle Park, NC,
May 19, 2004**

Panel Members

Henry Pitot, University of Wisconsin, Madison, Chair
Abraham Nyska, NIEHS
Lisa Kamendulis, Indiana University
Xi Huang, New York University
Hazel B. “Skip” Matthews, Matthews Consulting
Torka Poet, Battelle Pacific Northwest National Laboratories
Frank Welsch, Orbitox

Others in Attendance

Jeff Gift, National Center for Environmental Assessment, RTP
Tipton Tyler, Health Studies Management and Consulting
Bertram Price, Price Associates, Inc.
Chon Shoaf, National Center for Environmental Assessment, RTP

Panel Conclusions

The panel voted unanimously that enough information now exists to support an informed decision concerning the significance of the BAL metabolite to the formation of EGBE induced liver tumors.

The panel voted unanimously that the current information is adequate to support the mode of action described in the position paper for the EGBE induced formation of hemangiosarcomas in male mice and the potential relevance of this finding to humans.

The panel voted unanimously that the available information supports a nonlinear cancer assessment approach for the male mouse liver tumors observed following EGBE exposure, and therefore it is reasonable to expect that a lack of hemolytic effects in humans would prevent the formation of liver tumors in humans.

The panel voted unanimously that enough information now exists to support an informed decision concerning the significance of BAL genotoxicity to the formation of EGBE induced forestomach tumors.

The panel voted unanimously that the current information is adequate to support the mode of action described in the position paper for the EGBE induced formation of forestomach tumors in female mice and the potential relevance of this finding to humans.


The panel voted unanimously that the available information supports a nonlinear cancer assessment approach for the female mouse forestomach tumors observed following EGBE exposure and therefore making it reasonable to expect that a lack of hyperplastic effects in the region of gastroesophageal junction in humans would prevent the formation of gastroesophageal tumors in humans.

Respectively submitted,

J. Chris Corton, Ph.D.
Recorder

U.S. EPA Toxicological Review of Ethylene Glycol Butyl Ether

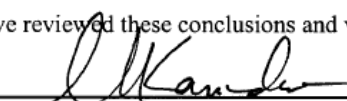
I have reviewed these conclusions and verify that they are correct.


Henry C. Pilot


Date

U.S. EPA Toxicological Review of Ethylene Glycol Butyl Ether

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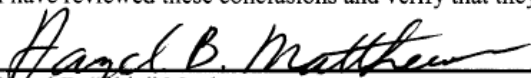
Lisa Kamendulis

6/16/04

Date


U.S. EPA Toxicological Review of Ethylene Glycol Butyl Ether

I have reviewed these conclusions and verify that they are correct.

 6.15.04
Hazel B. "Skip" Matthews Date

U.S. EPA Toxicological Review of Ethylene Glycol Butyl Ether

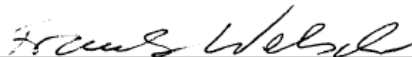
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Torka Poet

6/16/04
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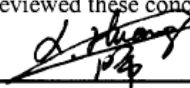
Frank Welsch



Date

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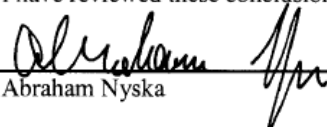
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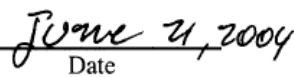

Xi Huang

6/17/04
Date

U.S. EPA Toxicological Review of Ethylene Glycol Butyl Ether

I have reviewed these conclusions and verify that they are correct.


Abraham Nyska


Date